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6. AUTHOR(S)
Karl Friedl, Stephen Grate, Susan Proctor

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Military Performance Division
US Army Research Institute of Environmental Medicine
42 Kansas Street
Natick, MA 01760-5007

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Over the past deade, the U.S. DoD invested >$150M to investigate undiagnosed Gulf War Illnesses (GWI) and more than twice that amount in clinical management and related efforts with regard to the same issues. The research produced important new understanding of post-deployment health issues and potentially hazardous occupational, materiel, and environmental exposures. Gulf War Illnesses issues also created a new awareness of important neuropsychological and neurotoxicological interactions which were not new problems, but which represented a difficult and relatively untapped frontier in biomedical research in chronic multi-symptom illnesses. Some GWI topics, such as blood-brain barrier integrity during stressful conditions and neurological effects of depleted uranium have been addressed, but others such as the neuroprotective benefits of aerobic exercise and psychosocial influences on individual stress resilience and resistance to neurotoxicity remain important areas of investigation. Current priorities for continuing investigation include: (1) practical neuropsychological test methods, (2) interactions between neurotoxic exposures and operational environments (e.g., exercise, heat, psychological stress), (3) structure-function relationships of neurotoxins with neurodegenerative disease potential, (4) objective correlates and biomarkers of neurological changes (e.g., neuroimaging with MRS), and (5) markers of individual susceptibility.

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Human subjects participated in these studies after giving their free and informed voluntary consent. Investigators adhered to AR70-25 and USAMRMC Regulation 70-25 on the Use of Volunteers in Research. For protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law CFR 46.

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BIOTECHNOLOGIES FOR ASSESSMENT OF TOXIC HAZARDS IN OPERATIONAL ENVIRONMENTS

BIOTECHNOLOGIES FOR ASSESSMENT OF TOXIC HAZARDS IN OPERATIONAL ENVIRONMENTS

Executive Summary

This group focused on markers of exposure for assessment of neurotoxicological threats from non-threat agents. Starting with reviews of standard approaches to toxic industrial chemicals and toxic industrial materials (TIC/TIMs), this group considered the specialized health risks to deployed military forces arising from exposure to toxic hazards chemicals that usually involve mixtures and interactions with other stressors and conditions. To narrow the discussion, two model systems were evaluated in detail, permethrin and JP8. These compounds represent militarily relevant chemical mixtures that are inhalation and dermal exposure hazards with neurotoxicological potential. Participating countries had various contributions to new research, evaluation, and discussion of approaches to assessing health and performance risks of these two categories of chemicals, ranging from neurobehavioral to special in vitro exposure test systems and cellular biomarkers. Interactions with physical factors (e.g., heat, dust, work/exercise), psychological stress, and other chemical exposures were evaluated. Communicating health risks to military forces to improve protective measures is itself a potential health risk and requires additional specialized research to establish rules of communication to achieve optimal compliance with safety and protective measures and minimal reductions. Two international EIHH workshops paralleled the efforts of this panel and expanded contributions to this work. Further work in these areas is being conducted with agreements to continue sharing of information on different approaches to assessing neurotoxicological risks. Recommendations were made for NATO and national implementation involving further development of efficient processes for early predeployment consideration of potential threats, assessment and monitoring of neurochemical hazards, and lifecycle health monitoring of exposed individuals.

Disclaimers:

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Chapter 9 – NEUROTOXICOLOGICAL INTERACTIONS WITH PHYSICAL AND PSYCHOLOGICAL STRESSORS[1]

Karl E. Friedl[1], Stephen Grate[1], & Susan P. Proctor[2]

[1]U.S. Army Medical Research and Materiel Command, Fort Detrick, MD, U.S.A. [2]U.S. Army Research Institute of Environmental Medicine, Natick, MA, U.S.A.

This chapter is based on a presentation "Lessons from the DoD Gulf War Illnesses Research Investment – Neuroepidemiology, Environmental Exposures, and Soldier Well-being" made at the NATO/PfP Workshop on Environmental and Industrial Health Hazards (EIHH) & Public Health Concerns in International Missions in Umea, Sweden, on 14 October 2004. A more comprehensive version is being prepared for publication.

9.1 ABSTRACT

Over the past decade, the U.S. DoD invested >$150M to investigate undiagnosed Gulf War Illnesses (GWI) and more than twice that amount in clinical management and related efforts with regard to the same issues. The research produced important new understanding of post-deployment health issues and potentially hazardous occupational, materiel, and environmental exposures. Gulf War Illnesses issues also created a new awareness of important neuropsychological and neurotoxicological interactions which were not new problems, but which represented a difficult and relatively untapped frontier in biomedical research in chronic multi-symptom illnesses. Some GWI topics such as blood-brain barrier integrity during stressful conditions and neurological effects of depleted uranium have been addressed, but others such as the neuroprotective benefits of aerobic exercise and psychosocial influences on individual stress resilience and resistance to neurotoxicity remain important areas of investigation. Current priorities for continuing investigation include: (1) practical neuropsychological test methods, (2) interactions between neurotoxic exposures and operational environments (e.g., exercise, heat, psychological stress), (3) structure-function relationships of neurotoxins with neurodegenerative disease potential, (4) objective correlates and biomarkers of neurological changes (e.g., neuroimaging with MRS), and (5) markers of individual susceptibility.

9.2 INTRODUCTION

9.2.1 The Problem

The controversies concerning undiagnosed illnesses following the Persian Gulf War deployment (1990-1991) stimulated many useful discussions, much research, and improvements in force health protection that will ensure better medical care for the returnees from future deployments. Among the most important advances are thoughtful approaches to linking undiagnosed symptoms with new operational threats through biomonitoring of individuals, monitoring the environment, methods to increase resilience to operational stressors, and imminent breakthroughs in preventive neurology, which will be based on better understanding of interacting threats affecting neurological outcomes. For Gulf War veterans who are ill, the research has not provided treatment for their problems; after concerted research efforts by the DoD (since 1994), there remains no clear link between the common symptoms (neuropsychological changes, chronic fatigue, and arthralgia)

[1] Authors: Friedl was the director of DoD GWI research program from 1994 to 2003; Grate has been the DoD technical expert for GWI and related neuropsychological and neurodegenerative disease research programs since 1997; Proctor is a key researcher in neuroepidemiology for the DoD, and previously conducted Gulf War Illness research at the Department of Veterans Affairs

[1] and presumptive exposures. The only difference between deployed and non-deployed troops in morbidity and mortality statistics was a 9% higher death rate, primarily attributable to motor vehicle accidents, and this excess mortality was dissipated after an additional seven year follow up [2, 3]. No new disease was discovered that was previously unknown to medical science, but there is a renewed emphasis on the still poorly understood family of disorders referred to as chronic multi-symptom illnesses [4, 5]. As a result of this research, other illnesses which may have occurred during of the deployment (e.g., Leishmaniasis), will be better understood. Safety evaluation of medical materiel has also been improved. It would be a surprising biological discovery if within a huge sample (~697,000 troops) there were zero adverse effects from the administration of a drug (e.g. pyridostigmine bromide), or vaccine (e.g., anthrax), or exposure to potent cholinesterase inhibitors such as DEET and permethrin. The question is, how do we identify at-risk individuals and how do we take safety to the next level, especially when casualties from enemy fire were extraordinarily low (372 deaths between Aug 1990-June 1991; 40% of these were combat casualties) and these wartime risks are actually lower than the potential risks associated with some of our own materiel.

Because no new syndrome was discovered, there is a perception that funding on Gulf War Illnesses research was money thrown into a black hole. In fact, many important findings and consequences have resulted from the spirited controversies produced by public discussion of poorly explained illnesses and from the resulting research. The DoD research to date has established new approaches to the early detection of changes in health status, new methods to monitor exposures, and advanced new understanding of how to assess the safety of medical prophylaxes and other materiel for use in operational environments (the interactions that go well beyond standard clinical testing for FDA approvals). Another important lesson is the appreciation of the impact of political and public pressures which can be supportive as well as hugely diverting when usually well-intended scientists enter the arena with extraordinary hypotheses to fill in the gap where research data is lacking. This huge gap in knowledge concerning neurological threats and outcomes is now better addressed by the DoD, and this should avoid a recurrence of the wide net of concerns that was thrown up in the absence of data on potentially harmful exposures and outcomes. These public concerns themselves produce adverse public health outcomes [6, 7]. Thus, the DoD's Gulf War Illnesses research program led to new discovery and emphasis on physical and psychological interactions affecting chronic multi-symptom illness; it addressed specific health risks and disease outcomes which had been previously overlooked; and it provided important lessons for the consequences of inadequate neurotoxicological data, biomonitoring of soldiers and environment, and health risk communication. These are all important themes of the TG-009 research group.

9.2.2 Reviews of Gulf War Illnesses Research

The health issues to be investigated and the research that was accomplished has been extensively reviewed by national committees and panels including the National Institutes of Health [8], the Defense Science Board [9], the Centers for Diseases Control [10], Institute of Medicine's committee on Health Consequences of Service During the Persian Gulf War [11] the Presidential Advisory Committee on Gulf War Veterans Illnesses [12], the Office of the Special Assistant for Gulf War Illnesses, the Presidential Special Oversight Board for Department of Defense Investigations of Gulf War Chemical and Biological Incidents [13] and a series of Rand Reports [14]. Congress directed that the DoD and the Department of Veterans' Affairs provide $0.5M to the Institute of Medicine annually between 1994 and 2003 to conduct epidemiological research on military and veteran populations. Many other investigator-led research reviews on Gulf War Illnesses have been published. In 1995, the first "working plan" for Gulf War research was published by the Persian Gulf Veterans Coordinating Board, composed of representatives from the DoD, VA, and HHS; this group coordinated federal efforts and published an annual report to Congress on Gulf War Illnesses research that continues today [15]. The DoD also funded several national conferences to facilitate

and accelerate the exchange of emerging research findings in federally funded Gulf War Illnesses research in Washington DC, June 17-19, 1998; June 23-25, 1999; and January 24-26, 2001.

9.2.3 Phases of Gulf War Illnesses Research Management

The research on Gulf War Illnesses progressed in roughly three phases. President Clinton directed that "no stone should be left unturned" in the investigation of the poorly defined illnesses of returning veterans. In the absence of a clearly defined pathology and with a wide range of potentially neurotoxic exposures, many different testable hypotheses were examined. Table 1 highlights the range of studies that gained considerable public attention and demonstrates some of the proposed solutions to these problems, particularly a mystery condition where there was no clear case definition and no specifically identified health risk. Many of these hypotheses in the table were investigated in DoD-supported research. In the second phase of the research, the focus was narrowed to most probable threats and outcomes. Health concerns had been better defined in a three-tiered medical examination of sick veterans in the Comprehensive Clinical Evaluation Program (CCEP), with many conventional diagnoses eliminated as a single prevalent problem [16-18]. The research in this second phase addressed many issues which had not been previously addressed for specific known threats and diagnoses. This also included some large-scale interventions that tested immediate treatments of undiagnosed disease (long term antibiotic treatment, and cognitive behavioral therapy), as well as hypothesized mechanisms of illness which would be determined by the treatment outcomes. In the third phase, the effort focused on chronic multi-symptom illnesses, and possible mechanisms based on psychological and physiological interactions that impair performance and produce disease.

Table I. Examples of Hypothesized Etiologies of Undiagnosed Symptoms of Gulf War Veterans.

• Multiple chemical sensitivities (or, toxic induced loss of tolerance, TILT) (Claudia Miller)
• Bacterial infection (Edward Hyman)
• Mycoplasma infection (Garth and Nancy Nicolson)
• Sarin exposure resulting in progressive brain damage (Robert Haley)
• Neurotoxicity of DEET and interactions with other pesticides (Mohammed Abou-Donia)
• Squalene antibodies caused by squalene adjuvant in vaccines (Pam Asa)
• Inhalation toxicology threats from depleted uranium (Asaf Durakovic)
• Burning semen syndrome (Jonathan Bernstein)
• Sand-borne illnesses (A. Korenyi-Both)

This chapter briefly reviews the status and key findings of the research efforts that were managed by the Army on behalf of the U.S. DoD as part of the Gulf War Illnesses research program (1994-2003), and the efforts that have continued forward as part of other programs such as the Force Health Protection (FHP) research program and the Neurotoxin Exposure Treatment Research Program (NETRP). Key lessons learned that are relevant to this NATO panel are the problems that arise in the absence of comprehensive monitoring of baseline health and deployment exposures: efforts to develop useful biomarkers of exposure and effect after the fact produce an unsatisfactory confusion between validation of the markers and determination of actual health risks.

9.3 SPECIFIC ETIOLOGIES AND DISEASES INVESTIGATED

9.3.1 Diagnostic Criteria for Infectious Diseases – Leishmaniasis

Leishmaniasis was an early contender in the search for etiological agents of a Gulf War Illness because it was known to be an endemic problem in some of the areas where U.S. forces deployed and because of its reputation as the "great masquerader", with elusive symptomatology mimicking other diseases [19]. After a relatively short period of excitement, including a brief ban on blood donations from all returning Gulf War veterans, the disease prevalence in returning soldiers was determined to be relatively low, with about two dozen cases of visceral Leishmania diagnosed [20]. Nevertheless, an entire program of studies was pursued with both extramural and intramural ($6M/4 years) efforts that culminated in a better understanding of the pathogenesis, vector control, diagnosis, and treatment of the disease. The program led to an advanced development effort to produce a commercially viable skin test which would be effective on both New and Old World Leishmania species and thus useful for future deployments in endemic areas of the world. Advances were made in the development of a serological test [21]. Funding to the basic and applied research program was terminated in 2002 to focus intramural research on higher priority endemic disease problems, although the problem of Leishmaniasis resurfaced with infections during more recent deployments to Afghanistan.

9.3.2 Other Infectious Disease Etiologies – Mycoplasma infection

Other theories of infectious disease etiologies were kept in the public view through popular press stories, Congressional testimony, and internet sites. Mycoplasma infection was postulated as a central etiological agent by a well-known cancer researcher, Garth Nicolson. He and his wife developed their own unique PCR assay technique and published data suggesting an association between infection rate in sick Gulf War veterans and their family members and substantial resolution of symptoms after antibiotic treatment [22]. The DoD funded the training of other mycoplasma investigators at the Nicolson lab and supported a blinded study with multiple labs assaying the same samples from sick and healthy Gulf War veterans. The results and investigator interpretations were reviewed by a seven member external panel that recommended that no conclusions could be drawn from this study regarding mycoplasma and Gulf War veterans [23]. Another theory addressed bacterial infection [24], with very high dose intravenous antibiotic treatment for 36 patients at the Truoro Infirmary in Louisiana. The findings of this study have never been accepted for publication and no report has been made publicly available to detail adverse consequences and benefits from the high dose treatments. Infectious etiologies as a basis of Gulf War Illnesses were investigated in a randomized controlled trial with a full year of antibiotic treatment (200 mg/d, doxycycline). This multi-site VA and DoD study used 491 veterans who had deployed to the Gulf, had at least two out of three of the symptoms of Gulf War Illness (fatigue, pain, and cognitive complaints), and who tested positive for mycoplasma DNA. There was no difference in outcomes of treated and untreated sick Gulf War veterans [25]. An effort to assess possible infectious agents used another biomoniotring approach in a study of 118 military working dogs that had deployed to the Persian Gulf. This study did not detect any new disease prevalence but did establish normal causes of death for two breeds, useful in future surveillance efforts [26].

9.3.3 Investigation of Neurotoxicity of Depleted Uranium

The depleted uranium (~60% less radioactive than naturally occurring uranium) used in some U.S. missile warheads, artillery and cannon rounds, and armor had never been assessed for health hazards to individuals on the receiving end of shrapnel and fragments. This became an immediate suspect in undiagnosed illnesses because of the perception that there might be a radiological risk, at least from fragments embedded in the tissues. Scientific questions were formulated around the neurological risks from heavy metal

and threats to renal function based on a well-known rodent model of acute renal failure that uses uranyl nitrate. Congress also entered into the discussion and outlined studies for the DoD investigating all aspects of safety associated with depleted uranium. Thirty three veterans known to have embedded particles of uranium based on high urine uranium excretion have been closely monitored and have not revealed any significant health consequences attributable to the slow dissolution of the embedded particles [27]. Small changes in neuropsychological status of injured veterans with embedded DU disappeared in later testing [28]. Several significant animal studies were conducted to test the carcinogenicity and the neurotoxicity of DU. Soft tissue sarcomas were noted at the highest levels of exposure, with very large pieces of DU implanted in the muscle of rats [29]. Although this response was comparable to positive controls using radioactive Thorotrast and not observed with foreign body comparisons using tantalum, rats are known to be much more sensitive to radiation carcinogenesis than humans, and the significance of the very high exposure conditions to veterans remains in question. Neurotoxicological investigations indicate that uranium from embedded pellets does distribute to nervous tissue but no adverse effects have been detected [30]. No significant human health risks have been associated with depleted uranium exposure; however, this continues to be a subject of public discussion [31, 32].

9.3.4 Ruling Out Teratogenic and Reproductive Effects – DoD Birth Defects Registry

Adverse reproductive outcomes, including fertility problems in men and women, teratological outcomes in the offspring of exposed individuals, and pediatric cancers, can be sensitive indicators of toxicological exposures. The DoD lacked a birth defects registry database for use in investigations of patterns of adverse outcomes and to be able to rule out possible associations and to reassure soldiers if there were no associations. Several studies investigated birth defects in the children of Gulf War veterans, both for factors which could be transmitted through the male and in pregnancies of women who had deployed to the Gulf. A condition with a difficult and often incorrect diagnosis, Goldenhar syndrome, became a target of investigation. Although it was a statistical challenge due to low prevalence of the syndrome, eventually it was determined that there probably was not a significant association [33]. Other epidemiological studies concluded that there was no significant increase in birth defects or in any other specific defect [34, 35]. Two studies that focused on depleted uranium effects transmitted through the male were inconclusive. Fertility problems are much harder to assess on a large scale unless there is a major effect and these studies require detailed clinical workup of male and female partners. The lack of a practical biomarker measure of male infertility is particularly challenging in such studies, where variability in semen sampling and multiple factors involved in assessing potency make conclusions about reproductive toxicity very difficult (e.g., aerosolized lead exposure studies in field artillery operations). [36]. Self reported pregnancy outcomes from female veterans of the Gulf War were similar to nondeployed women although this type of self report and voluntary participation study is inherently biased in favor of differences [37]. Testicular cancer was also investigated as a disease that has been commonly ascribed to greater risks with military deployments and certain occupational toxic chemical exposures. One pilot study using cancer registries suggested an increased prevalence [38]. Other analyses indicated a temporary increase, possibly related to deferment of care during deployment, with no difference in cumulative risks between deployed and nondeployed four years later [39]. Another condition, referred to as burning semen, briefly gained attention and was investigated but was rare and appeared to be no different than the frequency of this complaint in the civilian population as an immunological reaction that can occur in reproductive tracts of some individuals [40]. The Naval Health Research Center (San Diego, California) as a consequence of funded Gulf War research now maintains the DoD Birth and Infant Health Registry to monitor health of over 100,000 annual births to DoD personnel [41]

9.3.5 Searching for Adverse Effects of Pyridostigmine Bromide

Pyridostigmine bromide (PB) was distributed to US military forces in the Gulf War deployment to be used as a prophylaxis for the potential chemical warfare agent, soman. This was the first time that the drug had been used on such a large scale in healthy humans, although the actual useage rate was not well documented. As a cholinergic drug that had not been fully approved by the FDA for this application, this became an immediate suspect in the etiology of mystery neurological illnesses. Several important questions emerged that had not been specifically addressed in prior research including: PB penetration of the blood-brain barrier under high stress conditions, individual susceptibility to adverse effects of acetylcholinesterase inhibition; to include exposure of women (research had focused on effect in men) [42]; interactions with other compounds; interactions with other stressors such as physical stress [43]; and "bromism" from bromide accumulation. Extensive studies addressing each of these areas has substantially ruled out adverse effects of PB alone on the typical healthy individual but interactions with other compounds and stressors may be more complicated, as described in the next section Rare exceptions in biological responses would not necessarily be detected, but have also been described [44]. Standardization of cholinesterase inhibition assays has been challenging and remains an important priority to produce reliable measures of status of an individual following drug administration or chemical exposure [45-47].

9.3.6 Cholinergic Interactions – DEET, Permethrin, and Pyridostigmine Bromide

Pesticides were deployed for approved uses in the Persian Gulf deployment but these were individually approved uses for safety and efficacy and did not consider safety with more complicated interactions. Less well examined before the deployment was the interaction of chemical prophylaxes, other materiel, and other physical and psychological stressors simultaneously affecting the nervous system. In some cases, there could be direct interactions of the chemicals and stressors (e.g., altered dermal or inhalation routes of entry during heat or exercise exposures). Studies by Hermona Soreq suggested that acute psychological stress (forced swim stress in rats) might alter blood brain barrier access to pyridostigmine [48, 49]. This led to a flurry of studies on physiological alterations produced by relevant stressors, looking empirically at interactions in various combinations and doses, as well as carefully designed studies that tested proposed mechanisms of interactions. The reported effect of stress on blood-brain barrier integrity has not been well supported but important differences in interactions of organophosphorus pesticides at the metabolic level are emerging [50, 51]. Soreq's hypothesis was tested directly and no evidence has been produced to support the contention that there is a greater permeability in high stress conditions; however, effects can be enhanced by stress mechanisms through peripheral actions [52]. News headlines were made from reports that DEET might be more neurotoxic than previously recognized [53]. This claim depended in part on how brain histology was scored and relied on sensitive techniques that were not easily reproducible in the hands of other scientists. An important finding on the interaction of DEET and permethrin on dermal absorption [54] provided evidence for claims that toxicity of individual chemicals deemed below health hazard thresholds could act synergistically. Some empirical studies inferred interactions of neurotoxic chemical combinations (e.g. [55]). A study in rodents that attempted to recreate the psychological, physical, and neurotoxic chemical exposures relevant to worst case exposures in the Gulf deployment found only a major effect from jet fuel exposures in rodents [56, 57]. A study that attempted to reproduce stressor (psychological stress and exercise) and chemical exposures (PB, DEET, permethrin) in humans in the laboratory found no effects in their outcome measures [58].

9.3.7 Jet Fuel and other Petroleum Products and Combustion Products of Tent Heaters

Petroleum products have been repeatedly investigated for occupational safety concerns but new studies were conducted in response to the Gulf War medical issues Combustion products of unvented tent heaters were considered a possible source of illness and demonstrated some associations in self-reported exposures and pulmonary symptoms [59]. Some soldiers obtained their own tent heaters that were not intended for use in military tents without venting. A careful reproduction of a likely desert deployment scenario with actual vintage 1991 Persian Gulf deployed military tents and heaters, and using several conceivable fuels (kerosene, JP4), was established within a special clam shell enclosure to assess air flow, particulates and gases. The researchers concluded that, in the worst conditions, the first 20 minutes of warm up, the heaters produced high emissions of sulphates and air conditions equivalent to bad city air pollution [60]. Respiratory diseases were carefully assessed as part of the CCEP, especially because of the oil well fires that had been deliberately set during the war. No significant increase in pulmonary diseases or reduction in pulmonary function was detected. Careful assessments of the hazards by meteorologists and preventive medicine specialists, to include melding the data with a painstaking recreation of likely individual and unit exposures, as well as structured telephone interviews with a large sample of veterans, have not yielded any new information on health risks [61, 62]. A multistressor rat study that assessed combinations of chemicals and other relevant Gulf War stressors included JP4, the principal petroleum product used during the Gulf War. In all combinations, the groups receiving JP4, with or without other components, demonstrated significant neurotoxicological effects in a variety of tests in the Navy Toxicological Assessment Battery [56, 57]. Assessments of worst-case jet fuel exposure in certain job specialties in the Air Force, separate from the Gulf War Illnesses effort, have failed to produce any significant relationship to Gulf War Illnesses symptoms or any other important health effects [63]. Some neurological outcomes are of interest but have not been well addressed or fully analyzed in these previous studies. This has been discussed in the TG-009 work sessions and new studies were developed to attempt to address JP8 exposures and potential neurotoxicologic effects.

9.3.8 New Inquiries into Health Consequences of Low Dose Chemical Threat Agents

Sarin was carefully studied in the program after revelations that a bunker with sarin had been destroyed by combat engineers, possibly generating a low dose airborne exposure to thousands of troops in the surrounding region. Although there were no acute symptoms, including miosis, noted in troops at the time, new inquiries into short term, very low dose (sub-miosis thresholds) exposure effects on long term health outcomes have been investigated. As with DU, no careful health hazards assessments had been conducted on chemical threat agents that were intended to deter or kill an enemy. Several controlled animal studies examined low dose sarin exposures and interactions with other chemicals or deployment stressors, although several of these projects encountered major challenges in establishing suitable laboratory procedures for studies with sarin. Low levels of sarin did not interfere with thermoregulation in rat inhalation studies [64]. In the same series of studies, repeated low level exposures produced delayed reductions in acetylcholinesterase and muscarinic receptors in the olfactory bulb and other parts of the brain; with the addition of a stressor (heat strain), the hippocampus was also affected [65]. Studies with marmosets demonstrated EEG changes (increased alpha frequency sleep-spindles) more than a year after a 5 hour low level sarin exposure, and less pronounced changes with pyridostigmine pre-treatment [66]. Studies of some of the Tokyo subway sarin attack victims indicate sleep disturbances and memory deficits ten years later, suggesting a consistency with the animal observations. In an unusual role for the Institute of Medicine mandated by Congress, three large studies were conducted, one following up the long term health status of volunteers who had been deliberately exposed to chemical agents in studies at Aberdeen Proving Grounds in the 1970s [67], and another that examined morbidity and mortality of soldiers who had been in the vicinity of Khamisiyah during the demolition of the bunkers [68]; the third examined health care seeking behaviors of

veterans [69]. The first study found no unusual pattern of illnesses in previously exposed research subjects, the second, the Khamisiyah study, reported an increased risk in brain cancer deaths among those considered exposed, based on exposure estimates and geographical locations at the time of the demolition of the Khamisiyah chemical munitions bunkers. One theory advanced by Robert Haley was that reduced paraoxonase enzyme levels in some individuals might make them more susceptible to sarin neurotoxicity, explaining lower paraoxonase blood levels and brain damage in symptomatic Gulf War veterans [70]; these findings await confirmation.

9.3.9 Observed Changes in Immunological Status and Vaccine Associations

Approximately 150,000 soldiers received FDA-approved anthrax vaccine: several studies found no relationship between immunizations and chronic multi-symptom illnesses. Further investigations of anthrax vaccine safety have been conducted to ensure safety after an expansion of the vaccination program in 1998 intended to protect the entire military [71]. Hotopf et al. reported that administration of multiple vaccines during deployment produced significant multisymptom illnesses not observed in other individuals receiving multiple vaccines. They concluded that there may be a psychological stress effect during deployment which affects the responses to vaccines [72]. This was further pursued with mechanistic investigations, testing the hypothesis that stress caused a shift towards increasing "Th2" T cell cytokine profiles including increased interleukin-10 secretion. The data suggested that sick Gulf War veterans were characterized by a "Th1" cytokine profile [73]. Another theory derived from experience with silicone breast implant court testimony was that the symptoms of Gulf War illnesses were the result of immunological dysregulation associated with squalene antibodies. Dr. Pam Asa hypothesized that squalene antibodies would be higher in sick Gulf War veterans and were a consequence of the use of squalene as an adjuvant in vaccine production [74]. While squalene has been used as an adjuvant in some foreign vaccines, no Gulf War veterans were known to have been administered vaccines produced with squalene adjuvant; nevertheless, this concept was popularized in lay press articles and became another etiology to investigate. A study by the Naval Health Research Center compared the prevalence of squalene antibodies in sick and healthy Gulf War veterans and results will soon be reported.

9.3.10 Neurodegenerative disease risks: Amylotropic Lateral Sclerosis (ALS)

Two different studies of Gulf War veterans each indicate a two-fold increased risk of ALS for servicemembers who deployed to the Persian Gulf. In one study, 40 cases were found for 696,118 deployed veterans (0.67/100,000), compared to 67 cases in 1,786,215 non-deployed veterans (0.27/100,000); the greater prevalence of ALS was highest in Air Force and Army (compared to Navy and Marine Corps) [75]. Another study reported a two-fold greater prevalence in Gulf War veterans by comparing 20 diagnosed cases in veterans to literature values for the general population [76]. A key problem with such studies of diseases with extremely low prevalence is the problem of case ascertainment and the effect of relative small differences between the compared groups. Any disease in the highly studied deployed Gulf War population is more likely to be identified ("ascertainment bias"). On the other hand, the relatively young age for disease presentation in these cases has been suggested to support a relationship to deployment exposures. On the strength of the first study by Horner, the Department of Veterans Affairs determined that it would provide service-related compensation to the small number of veterans who had deployed and developed ALS. In other reports, Dr. Haley suggested that other neurodegenerative diseases such as Parkinson's would also be expected in higher rates in deployed Gulf War veterans [77]. To date, no such associations have been detected. There are strong associations between some neurotoxic chemical exposures and neurodegenerative diseases; however, currently available data are still relatively weak for any Persian Gulf deployment association and neurodegenerative disease. A retrospective study of older veterans compared military veterans to national

rates, finding no differences overall in Parkinson's disease but a higher rate for ALS [78]. A recent review of existing data by a panel at the Institute of Medicine concluded that there is an association between the Gulf War deployment and increased risk of ALS.

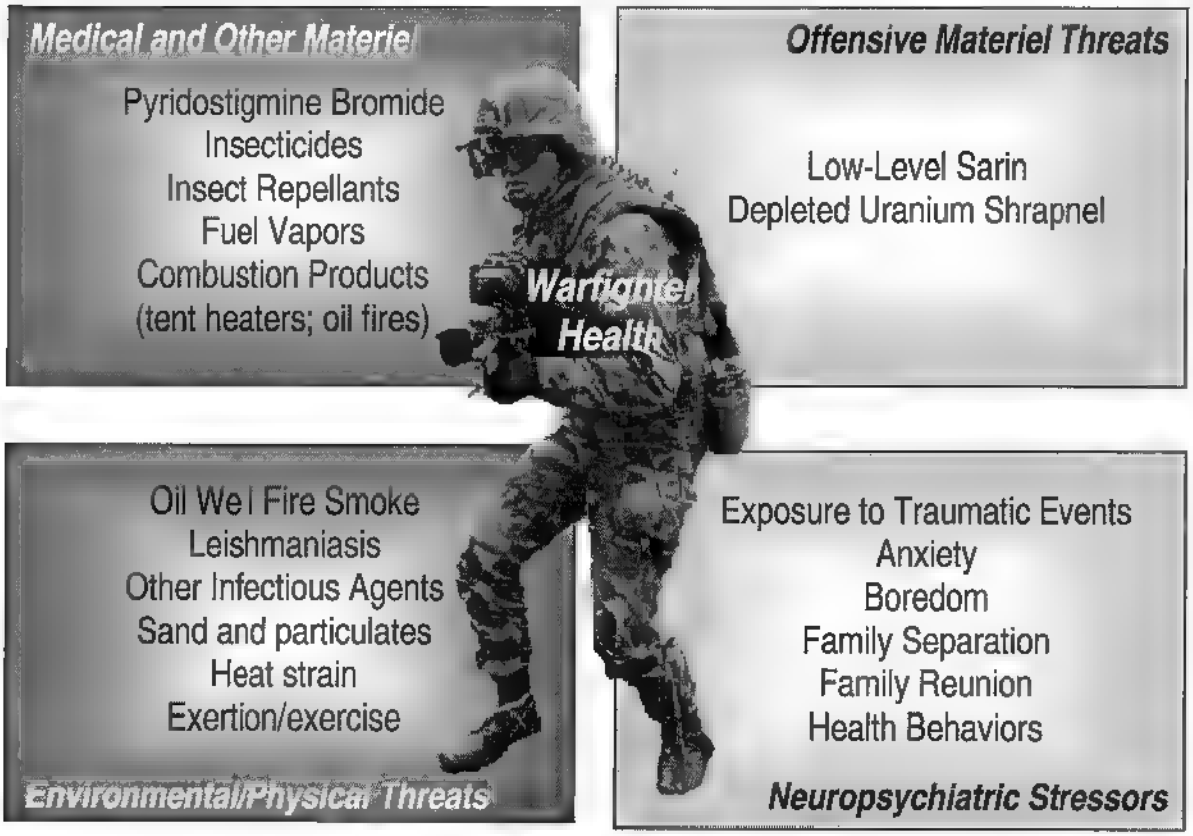

Figure 1. Examples of some of the multiple stressors surrounding soldiers during the Gulf War in 1990-1991. Warfighters are rarely subjected to one stressor at a time yet little of our research before the Gulf War had evaluated interactions relevant to military operational environments

9.4 CHRONIC MULTI-SYMPTOM DISEASE AND WELLNESS

9.4.1 Case Definition of a Poorly Defined Neurological Outcome

The first complex of symptom complaints that lacked a standard diagnosis emerged from a survey of an Army reserve unit by a young preventive medicine officer, Bob DeFraites [79], although they did not appear in standard diagnostic codes [80] Other studies quickly expanded the effort, including surveys focused on regional locations/specific states [81, 82] and military units such as the Seabees [83]. An Air Force survey by a CDC researcher, Fukuda, established a case definition of the most common undiagnosed symptom complexes which specified two out of three complaints of neurocognitive deficits, fatigue, and arthralgia [1]. The Fukuda definition is the one that is now most commonly used to classify otherwise undiagnosed but sick

Gulf War veterans as suffering from "Gulf War Illness," a category of poorly defined chronic multisymptom illnesses that include recognized diseases such as chronic fatigue syndrome and fibromyalgia. Eventually, the DoD-funded effort included several longitudinal cohort studies, notably, a Department of Veterans Affairs national survey [84], continued analysis of the DoD Gulf War registry population [62] a military sample centered at Fort Devens, Massachusetts [59], a state-wide military sample in Iowa [85], and a study of British forces [86]. The most important findings from these studies were that Gulf War era veterans who had actually deployed to the Gulf registered more complaints on all symptom checklists than individuals who did not deploy or other era comparison groups.

9.4.2 The Haley Hypothesis

Robert Haley published detailed studies of sick veterans based primarily on a set of symptom complexes he derived from one of six clusters in an epidemiological survey of sick veterans: "Haley Syndrome 2" (including detailed clinical studies of approximately 26 sick Gulf War veterans compared to a similar number of healthy comparison veterans who had deployed to the Gulf) [87, 88] His conclusion was that these illnesses represented a neurodegenerative disease involving damage to the right basal ganglia that he attributed to neurotoxic chemical exposures unique to the Gulf War deployment. This became a very public discussion when Ross Perot championed the concept and suggested that the DoD was overly focused on psychological stress rather than toxic chemical etiologies. A special independent panel on Gulf War Illnesses (the Presidential Special Oversight Board on Gulf War Illnesses) appointed by President Clinton reviewed the Haley data and concluded that the findings needed to be confirmed by other researchers. A large DoD-funded study, which has not yet been published (and which involved Dr. Haley as a consultant on the methodology) studied a new and larger sample of Gulf War veterans and included careful assessment of potentially confounding problems such as alcoholism, severe depression, and PTSD Another federal advisory panel, the VA Research Advisory Committee, was established by order of Congress to advise the Department of Veterans Affairs on further research in Gulf War Illnesses. Their most recent recommendation to the Department of Veterans' Affairs is a research solicitation for $15M/year to continue research led by Dr. Haley on topics primarily related to neurotoxic chemical etiologies and with specific exclusion of studies focused on psychological factors [89,90]. The publicity surrounding the Haley data directly contributed to a renewed effort by the DoD to investigate Gulf War medical issues through the establishment of a four year $20 million/year research effort on Gulf War Illnesses. Much of the research discussed in this chapter was funded as a result of this program. Unlike the focus of the VA RAC to prove a simple "smoking gun" etiological agent such as low-level sarin exposure, the DoD effort has diverged to multifactorial studies of chronic multisymptom illnesses, including both psychosocial factors and neurotoxic chemical exposures.

9.4.3 A Focus on Chronic Multisymptom Illness

Several researchers have exhausted their Gulf War study cohorts, finding that many veterans even after entering studies of illness claim to be better or recovered, but many Gulf War veterans still report symptoms of illness and these illnesses have lasted longer than those in veterans from other deployments [91]. The new emphasis on chronic multisymptom illnesses has led to some new understanding in this area, regarding the importance of activity and continued engagement following exposure to major life events such as a wartime deployment. Other Congressional special interest programs specifically supporting efforts in fibromyalgia and chronic fatigue syndrome have been linked to the Gulf War Illnesses focus in this area [92]. A treatment center at Walter Reed Army Medical Center has developed outcomes from the research to develop cognitive behavioural therapy approaches for symptomatic soldiers. A large study by VA and DoD on cognitive behavioural therapy of undiagnosed Gulf War veterans produced some apparent benefits [93]. Other

researchers have focused on sympathetic changes such as orthostatic hypotension [94], and other neurological indicators [95]. Specific odors and involvement of the olfactory system triggering biological responses (e.g., cadaverine in relation to exposure to psychologically traumatic events such as mass gravesites), and a wider variety of odors such as those that may trigger trigeminal responses through irritant and other chemesthetic pathways that may condition future neuroplastic responses, were also investigated [96-98].

While no new disease that was previously unknown to medical science has been discovered through the Gulf War Illnesses research effort, the many scientific discoveries and accomplishments (Table 2) have advanced military medical science and improved the ability to respond to future deployment health issues.

Table 2. Some Key Research Accomplishments of the DoD Gulf War Illnesses Research Program

- Ruled out numerous etiologies as likely causes of significant unexplained illnesses
- Developed Leishmania diagnostic skin test
- Determined that depleted uranium is less of a health hazard than initial concerns suggested
- Investigated stress effects on blood brain barrier integrity
- Refined safety assessments of pyridostigmine bromide for chemical agent prophylaxis
- Improved neurotoxicological testing methods
- Advanced knowledge of immune responses to vaccines in stressful environments
- Refined baseline testing such as predeployment health screens and neuropsychological testing
- Investigated safety of combinations of pesticides, jet fuel, pyridostigmine bromide, low level sarin, and other Gulf War deployment stressors, developing new understanding of important interactions
- Established new epidemiological cohorts and registries for more responsive investigation of future deployment and occupational health concerns
- Advanced the field of chronic multisymptom illnesses pathophysiology, diagnosis and treatment

9.5 NEUROTOXIN EXPOSURE TREATMENT RESEARCH

A related Congressionally-sponsored effort, the Neurotoxin Exposure Treatment Research Program (NETRP), began with $25M in 1997. This was intended as a Parkinson's research program but the investigation of Parkinson's Disease as both a neurological disease model and with pathogenesis directly relevant to military health threats is a very important extension of the efforts to correct and prevent issues identified in the Gulf War Illnesses from being experienced in future deployments. A study demonstrating low genetic concordance for Parkinson's Disease based on twins (compared to some other neurodegenerative diseases such as Alzheimer's that have a much stronger genetic basis), supported the notion that this could be an important model for the DoD to focus neuroscience research on, with dual use (military threats and civilian disease treatment) applications. Head injury, neurotoxicological threats including pesticides or nerve agents, chronic psychological stress, and traumatic stress are all relevant military stressors with neurological health and performance implications of great importance to the DoD, having common pathogenic mechanisms such as oxidative stress [99]. Many of the important projects underway today have direct military relevance and relevance to the efforts of this NATO panel including identification of biomarkers of early neurological changes. Projects concerning specific neurotoxic threats (pesticides including permethrin, PCBs, and methyl mercury) and fundamental studies on neural plasticity including mechanisms to accommodate changes in cholinergic function provide insight for development of enhanced protection of soldiers. An improved understanding of the pathogenesis of neurodegenerative diseases will allow the DoD to better protect soldiers by physiological monitoring and to specifically steer away from inadvertently harmful exposures. Neuroepidemiology provides an opportunity to test this understanding as it develops and to point the way for well-focused hypothesis-driven studies.

9.6 FORCE HEALTH PROTECTION RESEARCH, 2003+

The Defense Science Board recommended in June 1994 that there was insufficient evidence to support the concept of a new syndrome and that many veterans' symptom reports were similar to chronic fatigue syndrome. At the end of a decade of research into Gulf War Illnesses, this assessment has held up despite examination of virtually every testable hypothesis, no matter how unlikely or unpopular. In the course of this research effort, many related medical issues were solved or knowledge was markedly advanced, and the DoD has adopted a new emphasis on pre- and post-deployment health issues. Joshua Lederberg, the chairman of the task force on Persian Gulf War Health Effects, noted that "***high-tech, low-casualty campaigns in exotic places will engender a preoccupation with residual health effects as a fact of life for the foreseeable future. If chemical or biological weapons are ever actually employed, there will be a gross multiplication of those residuals (on top of obvious acute physical and psychological casualties), and further research is needed on long-term consequences of exposure.***" The panel reiterated the need for better pre- and post-deployment medical assessments coordinated between DoD and VA. The current focus of the U.S. Army Medical and Materiel Command (USAMRMC) Force Health Protection (FHP) research program is shown in Table 3.

Table 3. Current Force Health Protection (FHP) research, showing examples of research initiatives

- Identify fitness components affecting mission readiness
- Explore neuroprotective benefits of exercise
- Develop strategies to improve weight management in military environments
- Determine factors affecting effectiveness of educational interventions in basic training for health damaging behaviours
- Develop more effective psychological support systems for deployed family members and families
- Examine jet fuel exposure and neurological health in military personnel
- Assess permethrin exposure in operational conditions
- Monitor uncontrolled exposures through longitudinal prospective examination of military members (Millenium Cohort Study)
- Retrospectively study military occupation and neurodegenerative diseases
- Develop system to link surveillance data to health and fitness data
- Develop data mining capabilities for health outcomes derived from military electronic health records
- Develop a robust validated assessment battery to track neuropsychological health status of soldiers
- Assess neurocognition prospectively in future Gulf-deployed and Gulf-non-deployed soldiers

In 1999, three DoD Centers for deployment health were established to fulfill key roles in protection, detection, and treatment of disease threats in future deployments (Table 4).

Table 4. DoD Centers for Deployment Health

- Research: Center for Deployment Health Research (Naval Health Research Center, San Diego, California) (http://www.nhrc.navy.mil/rsch/department164/program.htm)
- Surveillance: Defense Health Surveillance Center (Defense Medical Surveillance System, Center for Health Promotion and Preventive Medicine, Aberdeen Proving Grounds, Maryland) (http://amsa.army.mil/)
- Treatment. Deployment Health Clinical Center (Gulf War Health Center, Walter Reed Army Medical Center, Washington, D.C.) (http://www.pdhealth.mil/)

The Center for Deployment Health Research includes the Millenium Cohort (MilCohort) study in its portfolio of center projects [100]. The MilCohort is a 21-year study of a stratified cohort of nearly 80,000 service members enrolled in 2001 (before the September 2001 attacks on the World Trade Center and the Pentagon) [101]. Two additional enrollment periods in 2004 (30,000 more enrollees) and 2007 expand the group to provide data on secular (temporal) trends. Data collection includes self-report surveys of the cohort every three years, combined with data from other records on occupational exposures, deployments, injury,

medications, health care utilization, disability, and mortality. The participants are being followed beyond their periods of military service. One of the first reports on neurological health focused on mental health status of service members after the September 11 terrorist attacks [102].

The data emerging from recent studies indicate the important interactive roles of psychological stress and neurotoxic chemical exposures. For soldiers returning from recent deployments in Afghanistan and Iraq, the primary concerns are about psychological stress and mild head trauma from impact or blast, distinguishing these two causes and examining their interactions to produce psychiatric casualties and longer term neurological sequalae. One of the needs identified out of the Gulf War experiences is a clearly defined neuropsychological baseline test. This has been developed with support from the Gulf War Illnesses research program, Force Health Protection research program, and the Neurotoxin Exposure Treatment Research Program. One recent study demonstrated changes during deployment that may reflect normal brain plasticity in response to the requirements of the environment, in this case with deployment to Iraq, a hypervigilance requirement during operations and persisting post-deployment may be the explanation for a post deployment increase in reaction time as well as a decrease in some other cognitive functions such as short term memory [103]. Further studies that include testing with neuroimaging and biochemical markers, as well as pathological changes in neurodegenerative diseases will continue to refine the testing and monitoring capability for neurocognitive function, a capability that did not exist during the first Gulf War.

9.7 CONCLUSIONS

In conclusion, the U S. DoD has invested a large amount of resources to investigate undiagnosed Gulf War Illnesses (GWI). Much of this expenditure could be avoided in future deployments with better characterization of exposures, pre-deployment investigation of health risks associated with likely neurotoxic exposures, and better tools to monitor exposures and health of individuals (i.e., biomarkers of toxic hazards). The Gulf War Illnesses research produced important new understanding of some of the relevant post-deployment health issues and potentially hazardous occupational, materiel, and environmental exposures. Gulf War Illnesses issues also created a new awareness of important neuropsychological and neurotoxicological interactions which were not new problems but which represented a difficult and relatively untapped frontier in biomedical research in chronic multi-symptom illnesses. Some GWI topics such as blood-brain barrier integrity during stressful conditions and the neurological effects of depleted uranium have been addressed, but others such as the neuroprotective benefits of aerobic exercise and psychosocial influences on individual stress resilience and resistance to neurotoxicity remain important areas of investigation.

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